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Induction of Dominant Mutations That Cause Skeletal Malformations in Mice

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A new approach for estimating genetic risk to humans from radiation is based upon an analysis of the frequency of induction of dominant mutations that cause skeletal abnormalities in mice. The main goal of this work is to improve estimates of the effect that an increase in the mutation frequency would have upon the incidence of serious genetic diseases in humans. The data obtained relate to dominant and irregularly inherited conditions in humans, which together constitute the great majority of human genetic diseases. The skeletal method could be used in chemical mutagenesis research in order to make a much more accurate risk-benefit analysis. A more likely application, however, is to provide a relatively quick and easy mammalian testing procedure for identifying mutagens. Dominant mutations at an unknown, but probably large, number of genetic loci could be detected. The relatively quick and easy procedure, which is described, has not yet been tested.

Most mutagenesis research is concerned with the question of whether a given chemical or physical insult can induce mutations and, if so, to what extent or by what mechanisms. With such data alone, it is very difficult to assess genetic risk. This paper describes a relatively new approach in mutagenesis research in which the emphasis is on the damage caused by the mutations. The endpoint recorded is the frequency of dominant mutations, detected in the first generation after treatment, that cause skeletal abnormalities in mice. Perhaps the biggest gap in knowledge required for estimating genetic risk to humans from radiation has been the lack of estimates of heritable damage in the first generation following irradiation in a mammal. The results described in this paper show an effective way to acquire such information.

Results of a large experiment by Selby and Selby are published elsewhere (*1*), and only a brief description will be given here. Male mice were exposed to 100 + 500 R of 60 R/min gamma radiation, with a 24-hr interval between fractions. After waiting long enough to ensure that the spermatogonial mutation frequency would be obtained, these mice were mated and their F_1 sons collected. The F_1 sons were raised and permitted to breed before they were killed and their skeletons were prepared for study. Alizarin-stained skeleton preparations were examined in detail through a dissecting microscope. If an abnormality suggested the presence of a mutation, some of the offspring of the F_1 were prepared for skeletal study in order to determine if the abnormality was transmitted. By means of this breeding test, it was possible to prove that abnormalities were caused by a dominant mutation. Furthermore, enough offspring of the F_1's were usually produced to enable mutant lines to be established for a more detailed genetical study.

Of the 2646 F_1's examined, 37 were concluded to be mutants. Of the 37, 31 were proved to be mutants by means of the breeding test and six, having no progeny, were concluded to be mutants on the basis of presumed mutation criteria. Almost all regions of the skeleton were affected by one or more of the mutations. The malformations recorded were those easily observable through a dissecting microscope. They consisted mainly of fusions of bones, too many or too few bones, and easily noticeable changes in the shapes or relative positions of bones. For three of the mutations, the only skeletal abnormality was a marked decrease in body size. Of the 31 proved mutations, 5 caused only one specific anomaly, 19 caused 2–5 anomalies, five caused 6–10 anomalies, and two caused 11–13 anomalies. Some of the anomalies were common to several different syndromes. Essentially all of the mutations have

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incomplete penetrance for some or all of the anomalies that they cause.

The mutations are described in detail in other papers (*2, 3*). Only some of the effects of one of the mutations will be described in this paper. This description will give a better idea of the types of effects caused by the dominant skeletal mutations. Mutation 320 causes all heterozygotes to have incomplete clavicles and a jagged edge at the anterior end of their nasals. In addition, almost all heterozygotes have a hole in the skull between the frontals. One effect which illustrates the occurrence of incomplete penetrance is that the interparietal bone is normal in 17% of the mice heterozygous for this mutation, but in 55% of the carriers it is divided into two pieces, in 24% into three pieces, in 3% into four pieces, and in 1% into five pieces. This mutation is especially interesting since it provides an excellent model of the autosomal dominant disease in humans called cleidocranial dysplasia.

Our experiment was patterned after the pioneering experiments of Ehling (*4*). The two main differences were that a breeding test was used to permit proof of transmissibility and a much larger sample was collected for a single radiation treatment. Ehling's experiments showed clearly that radiation induces a relatively high frequency of dominant mutations causing skeletal malformations. It is somewhat surprising that his results were not given more attention by those making risk estimates. This resulted, perhaps, from skepticism that his presumed mutations were mutations. The main question that our experiment was designed to answer was whether the types of malformations that Ehling thought were caused by dominant mutations were transmissible. Besides confirming Ehling's conclusion that radiation induces a relatively high frequency of dominant mutations, our experiment shows that at least most of Ehling's presumed dominant mutations must have been mutations.

There is a tendency for some nongeneticists to confuse our results with teratogenic effects. For this reason, it is important to stress the differences. The F_1 mice with malformations in our experiment were never irradiated; rather, it was their fathers which were irradiated. The malformations are inherited from generation to generation, demonstrating that they result from true mutations. As an illustration, if it is possible to maintain one of the mutations for 50 generations in the laboratory, it will still cause the malformations even though neither the mice showing them nor their ancestors for 50 generations have been irradiated. In contrast, if a mouse has a skeletal malformation of purely teratogenic origin (perhaps caused by irradiation during the period of major organogenesis), the descendants of this mouse in even the first generation would not have the malformation.

In an earlier paper (*1*), we suggested a way in which the mutation rate obtained in our experiment can be used to estimate the genetic risk to humans of having an induced dominant or irregularly inherited serious genetic disease in the first generation following increased exposure to radiation. Since these two categories of disease encompass about 95% of mankind's current load of serious genetic diseases (*5*), it is easy to see that the type of data accumulated using the skeletal approach has major implications for estimates of genetic risk.

It is worth noting, in more detail, why the data on dominant skeletal mutations in mice relate to dominant and irregularly inherited conditions in humans. Any mutations having complete penetrance, or almost complete penetrance, for recognizable malformations in heterozygotes would be considered dominants in any species. Mutations in mice having low penetrance for severe malformations in heterozygotes but causing no effects with complete or nearly complete penetrance that would be recognizable if they occurred in humans, would be equivalent to mutations in humans that cause severe effects that are presently thought to be irregularly inherited, but that are in reality the result of single dominant mutations with low penetrance. In humans, it would be much more difficult than in mice to recognize and confirm that a malformation is caused by a dominant mutation having incomplete penetrance. An unknown fraction of the irregularly inherited conditions in humans results from other modes of inheritance than dominants with incomplete penetrance; however, the skeletal approach relates to that fraction of the irregularly inherited conditions that would show a significant increase in incidence in the early generations following a change in the mutation frequency.

Our experiment was designed to measure overall damage to one body system caused by radiation-induced dominant mutations. Experiments of this type, involving time-consuming analyses of entire skeletons (about 25 min per F_1 individual) and breeding tests, will be used for making further refinements in estimates of genetic hazard to humans from radiation. It is important to consider what applications, if any, such a labor-intensive method might have for the assessment of genetic risk for the multitude of possible chemical mutagens. The improvement our data is making in knowledge about how an increase in the mutation frequency is related to an increase in genetic disease should be of great use to those facing this question for chemicals in the coming years. If a chemical of great potential usefulness to mankind were found to be a mutagen in

mammals by means of the specific-locus test in mice (6), further testing of the chemical in a skeletal mutation experiment of the type described above would permit a much more accurate comparison of benefit versus risk. The reason that such a test should be performed for that particular chemical is that the proportion of mutations induced by it resulting in malformations might be markedly different from the proportions found for mutations induced by radiation or by other chemicals. It seems likely that the skeletal method, in the form described so far, would only be used for mutagenic chemicals with potentially great benefits.

The skeletal method, in a modified form, may have much wider application to chemical mutagenesis research. The modification with the greatest potential is termed the sensitive-indicator approach. It derives its name from the suggestion in our experiment that a few specific malformations may be sensitive indicators of the presence of mutations. In our experiment, only three of the 2646 F_1's had an additional thoracic vertebra, and all three of these were proved to be mutants. Similarly, only three had a fusion of the triangular and pisiform, only two had the vertebra prominens shifted to the third thoracic vertebra, only two had a cervical rib. All seven of these mice were also found to be mutants. (Most of these 10 mutations cause additional malformations.) The finding that so few of the F_1's had any of these four malformations and that all mice with any of these malformations were mutants suggests that a large proportion, or perhaps all, of the F_1's with these malformations are mutants. Such malformations would be sensitive indicators of mutations. These data suggest that by using only a small number of sensitive indicators it may be possible to identify an important fraction of the total mutations that cause skeletal malformations. In our experiment, had we assumed that all F_1's having any of the four malformations listed were mutants, we would have found 10 of the 37 total mutations by that means alone.

In a sensitive-indicator mutation experiment, the incidence in the F_1 generation of only a small number of specific malformations, the sensitive indicators, would be determined for both a chemically treated group and its concurrent control. If the chemical did induce dominant mutations that cause malformations, the incidence of sensitive indicators would be much higher in the experimental group than in the control group. A sensitive-indicator experiment would be cheap and easy compared to the skeletal method used in making overall risk assessments. The time taken to examine a skeleton would be reduced by a factor of about 20; there would be no expensive and time-consuming breeding test; and the experiment could be performed by a technician having little training. Besides having these advantages, it would still be measuring dominant damage in a mammal resulting from mutations at an unknown, but probably large, number of genetic loci. This approach obviously has the potential of being of considerable usefulness in chemical mutagenesis testing.

It should be emphasized, however, that sensitive-indicator results would, at this time, be meaningless in the absence of a concurrent control. There is not yet enough evidence that the suspected indicator traits result solely from mutations. Such evidence can only be acquired as a by-product of further research of the type described in the first part of this paper. Furthermore, much work would be necessary to identify the malformations that are sensitive indicators in a different strain or under different laboratory conditions. In other words, much prior work is necessary in order to design a sensitive-indicator experiment that would have much chance of success. We hope to test this approach in the near future.

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